

Attorney Docket No.: 6056-236 (35926-136495)
AMENDMENT AND REPLY UNDER 37 C.F.R. § 1.111

THE AMENDMENTS

IN THE SPECIFICATION

Please amend the first paragraph on page 31 as follows:

The N-terminal sequence of native EC3 electroblotted onto PVDF membrane (Madsudaira, *J. Biol. Chem.* 262:10035-10038 (1987)) and residues 1-40 of cpEC3A and cpEC3B were determined by N-terminal sequence analysis using an Applied Biosystem Precise instrument. The primary structures of EC3A and EC3B were deduced from Edman degradation of overlapping peptides obtained by digestion with endoproteinase Lys-C (Boehringer Mainhem) (2 mg/ml protein in 100 mM ammonium bicarbonate, pH 8.3, for 18 h at 37°C using an enzyme: substrate ratio of 1:100 (w/w)) and CNBr (10 mg/ml protein and 100 mg/ml CNBr in 70% formic acid for 6 h under N₂ atmosphere and in the dark). Peptides were separated by reverse-phase of HPLC using a 0.4 x 25 cm Lichrospher LICHROSHPER RP100 C-18 (5 µm particle size) column (Merck) eluted at 1 ml/min with acetonitrile gradient. For determination of sulphydryl groups (free cysteines), native EC3 (2 mg/ml in 100 mM ammonium bicarbonate, pH 8.3 containing 6 M guanidine hydrochloride) was treated for 2 h at room temperature with 100-fold molar excess of iodoacetamide, dialyzed against distilled water, lyophilized and subjected to amino acid analysis (after sample hydrolysis with 6 N HCl for 18 h at 110°C) using a Pharmacia AlphaPlus amino acid analyzer.

Please amend the second paragraph on page 33 as follows:

Recombinant human VCAM-1 (0.5 µg/well) was immobilized in the wells of an ELISA plate overnight in PBS buffer. The plate was blocked using HBSS buffer containing 1% BSA. CMFDA-labelled Jurkat cells (1 x 10³ cells per sample) were added to the wells in the presence or absence of EC-3 in HBSS buffer containing 1% BSA. The plate was incubated for 30 minutes at 37°C. Unbound cells were removed by aspiration and the wells were washed with HBSS buffer. The bound cells were lysed with 0.5% Triton TRITON® X-100 (alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl)), and fluorescence was measured. Percent inhibition was calculated by comparing the fluorescence obtained for adhered

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cells in the absence (0% inhibition) and presence of EC-3. Figure 6 shows the percent inhibition with increasing concentrations of EC-3, in the presence (filled circles) or absence (open circles) of 1 mM Mn⁺⁺.

Please amend the second paragraph on page 38 as follows:

The RGDX RGD motif in monomeric disintegrins is replaced by MLDG (SEQ ID NO:14) in EC-3B. Both RGDX and MLDG may therefore represent integrin binding sites.